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The unique chiral structure of DNA has been a source of inspiration for the development of a new class of bio-inspired catalysts. The novel concept of DNA-based asymmetric catalysis, which was introduced only five years ago, has been applied successfully in a variety of catalytic enantioselective reactions. In this *tutorial review*, the ideas behind this novel concept will be introduced, an overview of the catalytic chemistry available to date will be given and the role of DNA in catalysis will be discussed. Finally, an overview of new developments of potential interest for DNA-based asymmetric catalysis will be provided.

Introduction

Enzyme catalysis is characterized by high catalytic activities and selectivities, achieved under mild conditions.[1] These attractive properties have been a source of inspiration for the design of synthetic catalysts.[2] Compared to synthetic catalysts, however, the catalytic repertoire of enzymes is limited. The emerging field of hybrid catalysis aims to bridge this gap and combine the catalytic power of transition metal catalysis with the chiral architectures of biopolymers such as proteins and DNA, with the ultimate goal of creating new catalysts that combine the best of both worlds.

A hybrid catalyst comprises a synthetic catalyst, often a transition metal complex, that is anchored to a chiral biomolecular scaffold.[3] Anchoring can be achieved *via* a covalent bond, but also supramolecular and dative approaches can be used. An attractive feature of hybrid catalysts is that the transition metal catalyst and the biomolecular scaffold can be optimized independently by chemical and genetic approaches.[4,5]

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Using proteins as the scaffold, a series of highly enantioselective catalytic transformations have been reported.[6–11] The progress in the field of protein-based hybrid catalysis has been described in a number of reviews.[12,13] In this tutorial review we will focus on DNA as the biomolecular scaffold, with a particular focus on the novel concept of DNA-based asymmetric catalysis.

DNA has several features which make it a very attractive chiral scaffold for hybrid catalyst design. It is chemically stable and has a well-defined chiral structure. The iconic right handed double helix of B-DNA is the predominant conformation, but depending on the hydration of the grooves, the ionic strength of the solvent, and the presence of DNA-binding molecules, other structures, including left-handed Z-DNA are accessible as well. Moreover, using the simple rules of Watson–Crick base pairing other three-dimensional architectures are available in addition to the ubiquitous double helix.[14,15] Finally, DNA is commercially available from both synthetic or natural sources; DNA from natural sources, such as salmon testes (st-DNA) and calf thymus DNA (ct-DNA) can be obtained in large quantities at cost prices that are in the range of those of small molecule catalysts.

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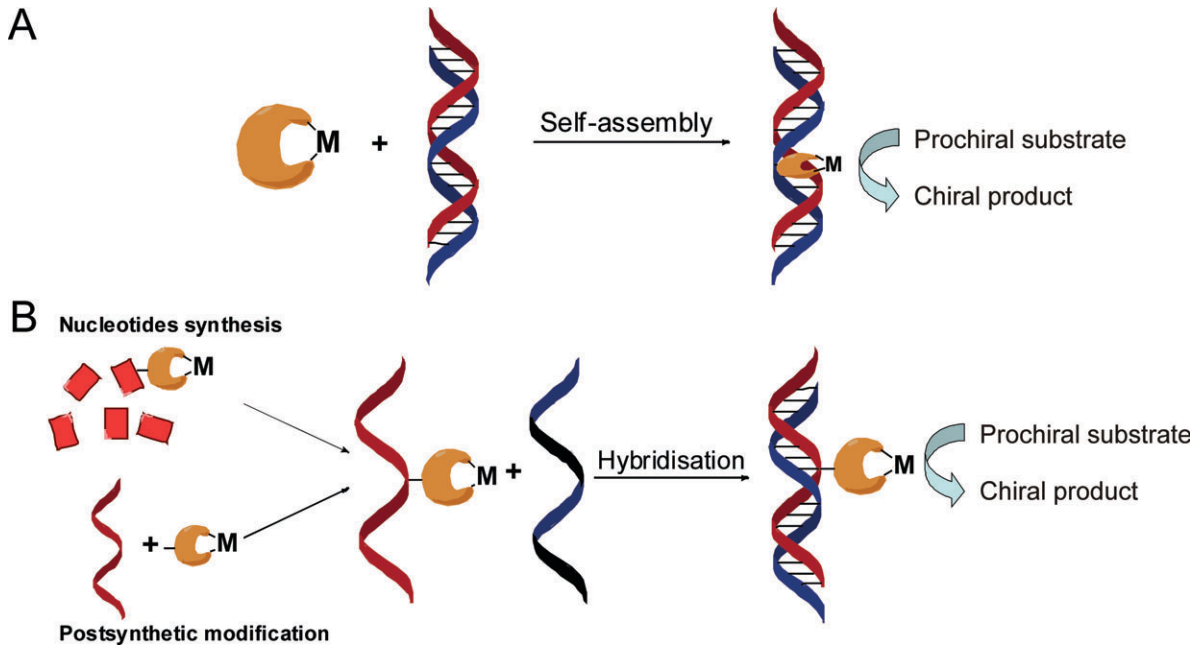
Arnold J. Boersma was born in 1979 in Bergum, The Netherlands. He obtained his MSc in organic chemistry, in 2005, at the University of Groningen. He received his PhD degree under the guidance of Prof. Ben. L. Feringa and Dr Gerard Roelfes at the University of Groningen, in 2009 on the topic of DNA-based asymmetric catalysis. He is currently undertaking a postdoctoral research fellowship in the group of Prof. Hagan Bayley at the University of Oxford, England.

Rik P. Megens

Rik P. Megens was born in Harderwijk, The Netherlands, in 1984. In 2007 he obtained his MSc in chemistry from the University of Groningen with specialization in organic chemistry. Currently he is working as a PhD student in the group of Dr G. Roelfes on DNA-based catalysis.



Scheme 1 Schematic representation of DNA-based asymmetric catalysis using the supramolecular (A) and covalent (B) anchoring strategies.

Two observations in the literature suggested the feasibility of using DNA as a source of chirality in catalysis. The first one being that DNA had been used in a variety of stoichiometric chemical reactions, resulting in diastereoselectivity and enantioselection of chiral substrates.[16] Furthermore, one case of enantioselective Diels–Alder reactions catalyzed by an RNAzyme has been reported.[17]

The concept of DNA-based asymmetric catalysis

A DNA-based catalyst comprises a transition metal complex based on a non-chiral ligand that is brought into close proximity of the DNA helix. As a result the catalyzed reaction takes place in, or very close to, the DNA helix, which allows the chirality of DNA to be transferred onto the reaction, resulting in products that have an excess in one of their enantiomers.

Two general strategies can be distinguished which rely on either supramolecular (non-covalent) or covalent anchoring of the transition metal complex to the DNA (Scheme 1). The non-covalent or supramolecular anchoring strategy is based on the propensity of DNA to bind small molecules using hydrophobic, π-stacking, electrostatic and/or hydrogen bonding interactions, resulting in intercalation and/or groove binding. Supramolecular anchoring can be achieved by incorporation of a DNA-binding moiety in the design of the ligand for the metal centre. Covalent anchoring involves binding of a transition metal complex *via* the ligand to the DNA using a small

Ben L. Feringa

Ben L. Feringa obtained his PhD degree in 1978 at the University of Groningen under the guidance of Professor Hans Wynberg. After working as a research scientist at Shell, he was appointed Full Professor at the University of Groningen in 1988 and named the distinguished Jacobus H. van't Hoff Professor of Molecular Sciences in 2004. He was elected foreign honorary member of the American Academy of Arts and Sciences, and member of the Royal Netherlands Academy of Sciences (KNAW). In 2008 he was appointed as Academy Professor of the KNAW. His research interests include stereochemistry, organic synthesis, asymmetric catalysis, molecular switches and motors, self-assembly and nanosystems.

Gerard Roelfes

Gerard Roelfes obtained his MSc from the University of Groningen, where he also completed his PhD in 2000, under the guidance of Prof. Ben L. Feringa, in a collaborative project with Prof. L. Que, Jr, University of Minnesota. He then went for a post-doc with Prof. Donald Hilvert at the ETH-Zürich. In 2003 he returned to the University of Groningen as a junior project leader. Currently, he is Assistant Professor of Biomolecular Chemistry. The central theme in his research is developing new bio-inspired concepts in catalysis. The main research topics in the Roelfes group are DNA-based asymmetric catalysis, modular assembly of novel DNA-based (catalytic) systems and semi-synthetic proteins, with a particular focus on artificial metalloenzymes.

spacer moiety. Attachment sites in this case can be modified nucleobases or phosphate esters.

Both these anchoring approaches have specific advantages and limitations. In the case of supramolecular assembly, the advantage is the ease of formation; the catalyst is spontaneously self-assembled by combining the transition metal complex with the DNA, which allows for rapid optimization. However, the catalyst generally will not be very well defined; it is likely that the transition metal complex binds at multiple positions to the DNA. As a consequence, the DNA-based catalyst is actually a heterogeneous mixture of different catalysts that reside in a different micro-environment. In contrast, covalent attachment allows for precise control over the positioning and, hence, structure and geometry of the catalyst. However, covalent modification of DNA is not straightforward and is very time consuming, which complicates optimization.

Supramolecular anchoring

The non-covalent or supramolecular anchoring strategy involves incorporation of a DNA-binding moiety in the design of the ligand. To date, two general designs of ligand have been used (Fig. 1). In the first generation of ligands, a DNA binding moiety is attached *via* a short spacer to a metal binding moiety.[18] The well known intercalator 9-amino acridine has been used as the DNA-binding moiety and aminomethyl pyridine constitutes the metal binding moiety. The substituent [R] and the length of the spacer [*n*] can be varied to optimize the design. In contrast, in the second generation of ligands the DNA-binding moiety is integrated into the metal binding moiety, which eliminates the need for a spacer. This class of ligands includes dipyrido[3,2-*a*:2′,3′-*c*]phenazine (dppz, **L5**), dipyrido[2,2-*d*:2′,3′-*f*]quinoxaline (dpq, **L6**), phenanthroline (phen, **L7**), bipyridine derivatives such as 2,2′-bipyridine (bipy, **L8**) and 4,4′-dimethyl-2,2′-bipyridine (dmbipy, **L9**).[19] Compared to the first generation of ligands, the catalytically active metal centre can be brought much closer to the DNA.

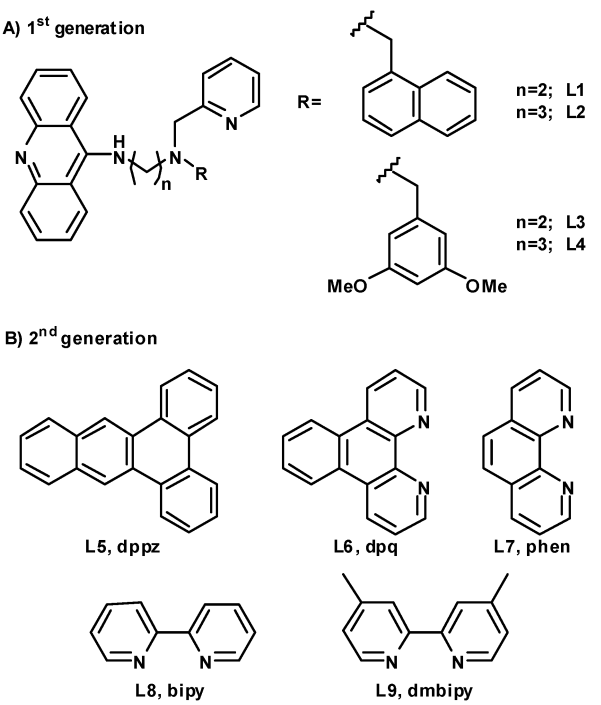
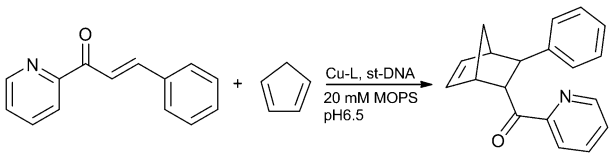


Fig. 1 First (A) and second generation (B) ligands used in DNA-based asymmetric catalysis.



Scheme 2 Cu^{2+} catalyzed Diels–Alder reaction of aza-chalcone with cyclopentadiene.

The catalysts are formed spontaneously by self-assembly of the corresponding copper complex with salmon testes DNA (st-DNA). The DNA-based catalysts were evaluated in the Cu^{2+} catalyzed Diels–Alder reaction of aza-chalcone with cyclopentadiene, which is a convenient model reaction (Scheme 2).[20] This reaction was selected for a number of reasons. It is well established that an aqueous environment is beneficial for the Diels–Alder reaction.[21] Furthermore, analysis of the reactions catalyzed by biomolecular catalysts such as RNAzymes,[17,22] DNAzymes[23] and catalytic antibodies[24,25] shows that the Diels–Alder reaction is in many cases a preferred reaction. This is most likely related to the fact that in a Diels–Alder reaction, going to the activated complex, large structural changes are occurring that are sensitive to interactions with the biomolecular scaffold. Finally, the present reaction, discovered by the group of Engberts, represents the first example of a Lewis acid catalyzed enantioselective reaction in water with ee's of up to 74%.[26] In the reaction aza-chalcone binds to the Lewis acidic Cu^{2+} in a bidentate fashion, which results in activation of the alkene for the Diels–Alder cycloaddition reaction. Dissociation of the product regenerates the catalyst, which is now available for a new cycle. The Diels–Alder product is obtained as a mixture of *endo* (major) and *exo* (minor) isomers.

The results obtained with the first generation ligands **L1–4** showed that the enantiomeric excess obtained is highly dependent on the design of the ligand employed. Key determinants of the enantiomeric excess and the enantiomeric preference, that is, which enantiomer of the product is obtained in excess, proved to be the nature of the sidechain [R] and the length of the spacer [*n*]. It was established that R preferably is an arylmethyl group, with the highest enantioselectivities obtained for R = 1-naphthyl methyl; up to 49% ee of the (−) enantiomer using **L2**. The need for an aryl group has been attributed to the importance of π–π stacking interactions with the bound substrate. Interestingly, using **L3** in which R = 3,5-dimethoxybenzyl, 37% ee of the opposite enantiomer (+) was obtained. In general, the best ee's were obtained when a short spacer of $n = 2$ or 3 was used. Elongation of the spacer resulted in a rapid decrease of enantioselectivity, showing that intimate contact of the copper complex with the DNA is required. Often opposite enantiomers were found when using $n = 2$ (**L1**) or $n = 3$ (**L2**). The fact that the enantiomeric outcome of the reaction can be controlled by judicious design of the ligand represents a particularly attractive feature of the

first generation ligands, since natural DNA is available in one enantiomeric form only.

The observation that the ee induced by the first generation ligands are higher with smaller spacers, suggested that using a complex of a ligand that does not require a spacer might further increase the enantioselectivity. For this reason the second generation of ligands was introduced.[19] Indeed, it was found that with these ligands significantly higher ee's were obtained, ranging from 49% with Cu(dppz) to 90% ee for Cu(bipy). The ee could be increased even further by placing methyl groups at the 4 and 4′ positions of the bipyridine. In this case an *endo* : *exo* selectivity of >99 : 1 and excellent ee of 99% for the *endo* isomer of the Diels–Alder product was obtained. A direct effect of these two methyl groups on catalysis can be excluded, since these are located at a distant position from the site of catalysis. More likely, the methyl groups alter the geometry of the substrate–catalyst complex bound to DNA such that a higher enantioselectivity is achieved. Interestingly, Cu(dmbipy) (**L9**) has only a moderate binding affinity for DNA; the binding constant (K_b) was measured to be $1.12 \pm 0.02 \times 10^4$ M^{-1}. This means that under catalysis conditions, that is low millimolar concentrations of DNA (in base pairs), not all of the copper complex is bound to the DNA.

Scope of DNA-based asymmetric catalysis

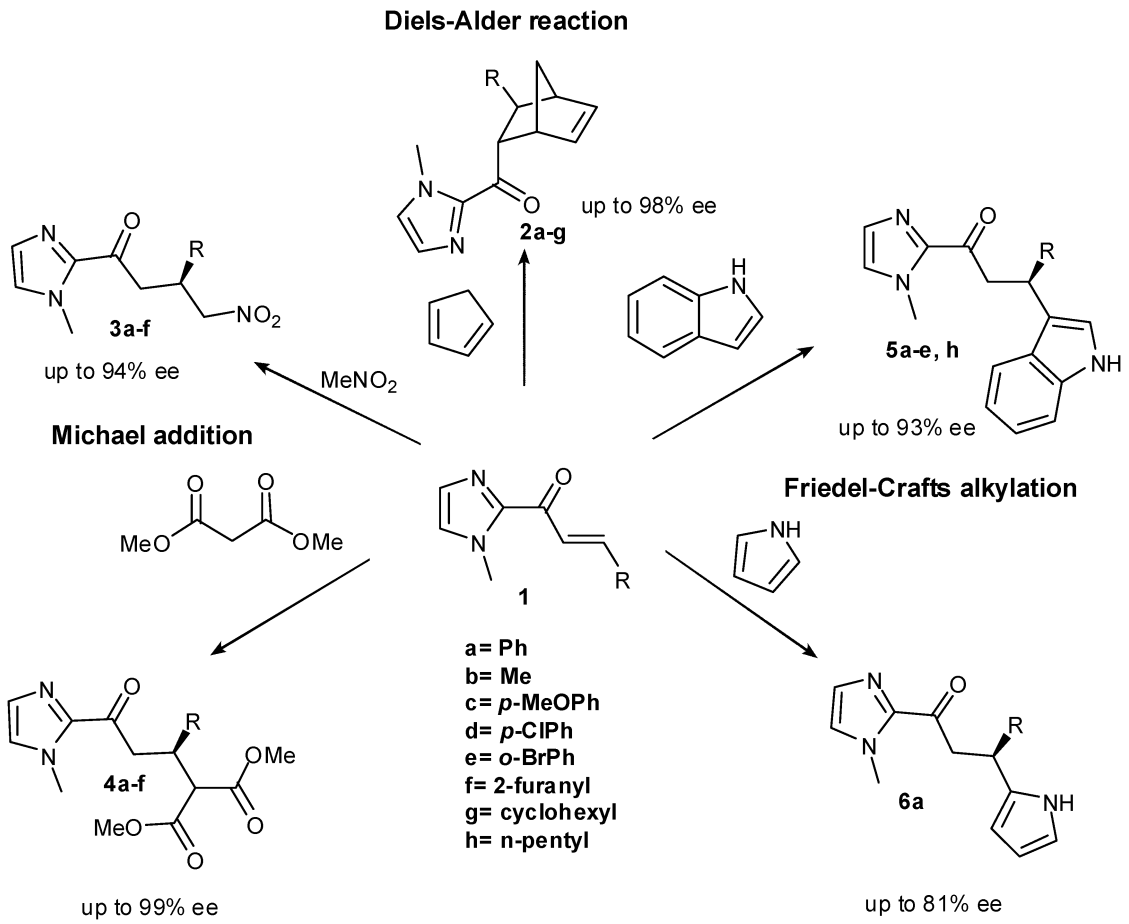
Once the concept of DNA-based catalysis had been demonstrated, the catalytic scope was investigated with a focus on the st-DNA/Cu-dmbipy catalyst, which generally gave rise to the best results in catalysis.

C–C bond forming reactions

The power of the DNA-based asymmetric catalysis concept was demonstrated in several of the archetypical C–C bond forming reactions, such as the Diels–Alder,[27] Michael addition[28] and Friedel–Crafts alkylation reactions (Scheme 3).[29] A key advance was the use of α,β-unsaturated 2-acyl imidazoles as alternative substrates to the aza-chalcones. The advantages of this class of substrates, introduced by Evans and co-workers,[30] include a straightforward preparation and facile removal of the imidazole group after the catalytic reaction, allowing for further synthetic transformations.

In the first example, α,β-unsaturated 2-acyl imidazoles were used as dienophiles in the st-DNA/Cu-dmbipy catalyzed Diels–Alder reaction with cyclopentadiene. Enantioselectivities of up to 98% were obtained, which is similar to the values achieved with aza-chalcone. The catalyst proved to tolerate a large variety of substituents at the β-carbon, ranging from aromatic to alkyl groups or even only hydrogen. In all instances the Diels–Alder products were obtained with ee's ranging from 80–98% (Scheme 3).[27]

Next, the potential of α,β-unsaturated 2-acyl imidazoles to act as Michael acceptors was evaluated in the st-DNA/Cu-dmbipy catalyzed Michael addition reaction in water.[28]



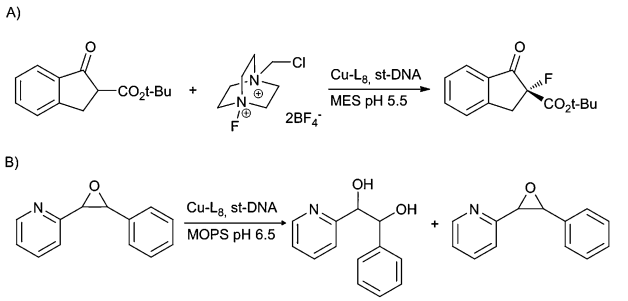
Scheme 3 Scope of st-DNA/Cu-dmbipy catalyzed C–C bond forming reactions.

Before, only scattered reports of catalytic asymmetric Michael additions in water were available, with a maximum ee of 86%.[31] Using dimethyl malonate as a Michael donor, the corresponding conjugate addition products were obtained in up to 99% ee (Scheme 3). Nitromethane was also employed as nucleophile, resulting in ee's of up to 94% for the Michael adduct (Scheme 3). These ee's represent the highest that have been obtained in catalytic asymmetric Michael additions in water to date.

A particularly interesting subset of conjugate additions is the reaction of α,β-unsaturated 2-acyl imidazoles with neutral π-nucleophiles such as indoles or pyrrole. These heterocycles are a recurring motif in many chiral natural products. From the perspective of the nucleophile this reaction is classified as a Friedel–Crafts alkylation. Traditionally, this type of reaction is associated with anhydrous conditions. Using st-DNA/Cu-dmbipy in water with substrate **1b** the corresponding Friedel–Crafts products could be obtained with ee's of up to 93% (Scheme 3); the first asymmetric Friedel–Crafts reaction in water.[29] The ee obtained depends on the β-substituent, but in contrast to the Michael and Diels–Alder reaction, the highest ee's were not obtained with aryl substituents but were obtained with alkyl substituents at the β-position. In the case of **1b** the catalyst loading was lowered to 0.3 mol% (2 mol% DNA in basepairs), giving rise to 330 turnovers, without observing a decrease in ee, even though under these conditions only 16% of the Cu-dmbipy is bound to the DNA, which amounts to an effective catalyst loading of only 0.05 mol%.

The reactions described have been carried out at a preparative scale. The conversion and the enantioselectivity did not change significantly when the scale was increased, although for the Michael addition longer reaction times were needed to achieve full conversion. Also, in all cases the catalyst loading could be reduced significantly: the Michael addition and the Diels–Alder reaction have been performed with 5 mol% copper, and in the case of the Friedel–Crafts reaction the catalyst loading could be reduced to 0.3 mol%. An additional advantage of DNA-based catalysis is that after the reaction the aqueous catalyst mixture is recycled readily; upon extraction of the products with Et_2O, fresh reagents were added to the aqueous phase containing the DNA-based catalyst and the reaction was continued. In this manner, the catalyst solution was recycled several times, each time providing similar isolated yields and enantioselectivities of the product.

Recently, it was found that water-miscible organic co-solvents can be added to the reaction without loss of enantioselectivity.[32] However, the reaction rate is affected: in some cases such as the Diels–Alder reaction the reactions are slower in the presence of organic co-solvents, whereas the Michael addition and Friedel–Crafts alkylations are generally faster, albeit with some exceptions. The tolerance for organic co-solvents allows for using poorly water-soluble substrates and increasing substrate concentrations. Moreover, by judicious choice of solvent, the reactions can be run at temperatures < 0 °C, which results in higher enantioselectivities. This is illustrated by the DNA-based catalytic Friedel–Crafts alkylation of **1b** with 5-methoxyindole, which was performed on the gram scale, using 30% v/v MeOH, at −18 °C, giving rise to 85% isolated yield of the corresponding product with an ee of 93%.



Scheme 4 Other enantioselective reactions catalyzed by st-DNA/Cu-dmbipy: (A) electrophilic fluorinations and (B) hydrolytic kinetic resolution of epoxides.

Other reactions

The group of Toru has successfully applied the st-DNA/Cu-dmbipy system in electrophilic fluorination reactions (Scheme 4).[33] In these reactions a β-ketoester binds to the Cu(II) centre, resulting in formation of the enolate, which then reacts with an electrophilic fluorine source such as Selectfluor®. The reaction proved to be very sensitive to structural variations in the β-ketoester; depending on the structure of the substrate, enantioselectivities varied from negligible to 74% in case of indanone carboxylates containing a bulky ester substituent.

The st-DNA/Cu-dmbipy catalyst has also been employed in the hydrolytic kinetic resolution of pyridyloxiranes.[34] The best results were obtained in the case of *trans*-β-phenyl pyridyloxirane where an *S*-factor of 2.7 was found. This selectivity is still far from what is required for practical applications, but it does demonstrate the potential of the DNA-based catalyst for kinetic resolution.

The role of DNA in catalysis

In the catalyzed reactions, DNA is the only source of chirality present. Based on this, it can be concluded that the enantiomeric excess observed in the reaction product is the result of the close proximity of the catalyst to the DNA. However, an important question is whether the DNA also influences the reaction itself. For this purpose a kinetic study of the catalytic Diels–Alder reaction of aza-chalcone with cyclopentadiene in the presence and absence of DNA was undertaken. With the first generation of catalysts the reaction proved to be slower in the presence of DNA.[35] In contrast, an acceleration was found using the second generation catalysts in combination with DNA.[36] This acceleration was modest in the case of Cu-bipy and Cu-phen, that is, a 2–3 fold increase in the apparent second order rate constant (k_{app}) for the reaction. Surprisingly, however, using Cu-dmbipy/DNA a 58-fold increase in reaction rate compared to Cu-dmbpy alone was observed. Hence, the reaction is DNA-accelerated.

A more detailed analysis revealed that the rate acceleration is a kinetic effect; it is the k_{cat} of the actual Diels–Alder reaction that is increased by 2 orders of magnitude; the binding constant K_a for binding of the aza-chalcone to the Cu is not significantly affected. Based on the ee values, the k_{cat} was separated into the $k_{cat(+)}$ and $k_{cat(-)}$, which are the rate constants towards the major and minor enantiomer, respectively. Interestingly, it was found that the observed rate

Table 1 Kinetic parameters of the 1st and 2nd generation catalysts

	Cu-**L3**		Cu-dmbipy	
	w/o DNA	With DNA	w/o DNA	With DNA
k_{app} (M^{-1} s^{-1})	0.031	0.026	0.0069	0.4
Acceleration		0.84		58
K_a (M^{-1})	1.8×10^4	8.2×10^3	4.0×10^2	5.0×10^2
k_{cat} (M^{-1} s^{-1})	0.11	0.1	4.5×10^{-2}	3.8
$k_{cat(+)}$ (M^{-1} s^{-1})	—	—	2.2×10^{-2}	3.8
$k_{cat(-)}$ (M^{-1} s^{-1})	—	—	2.2×10^{-2}	5.8×10^{-2}

[Cu-L] = 0.10–0.25 mM, 25.0 °C, MOPS buffer (20 mM, pH = 6.5), 6.0 μM aza-chalcone, 0.5–2.0 mM cyclopentadiene.

acceleration is fully due to an increase of the rate towards the major enantiomer; $k_{cat(-)}$ was found to be similar to the k_{cat} in the absence of DNA (Table 1).

The DNA sequence and, related to this, the DNA structure are important parameters in DNA-based asymmetric catalysis, as they determine the structure of the micro-environment in which the copper complex resides. St-DNA is composed of long pieces of variable length and, from the perspective of DNA-based catalysis, can be considered to have a random sequence. In view of the high loadings of copper complex used, the st-DNA/Cu-L catalyst is actually a heterogeneous mixture of catalysts that all provide a different micro-environment for the catalyzed reaction. In order to establish the importance of this micro-environment, a series of self-complementary oligonucleotides of defined sequence was evaluated in the catalysis. The sequence dependence was studied for both generations of ligands, in particular **L3** and dmbipy. In both cases a strong dependence of the enantioselectivity on the DNA sequence was observed. Using **L3** the best results were obtained using alternating GC sequences; the ee value increased from 37% for st-DNA up to 62% in the case of poly(dG-dC). In contrast, AT rich sequences gave rise to a strong decrease in ee. The second generation catalyst Cu-dmbipy showed a different sequence dependence. Now alternating GC sequences gave rise to a significantly lower ee compared to st-DNA, while sequences containing G tracts gave the best results (Table 2). The highest enantioselectivity was found with $d(TCAGGGCCCTGA)_2$, that is 99.4% ee.

An intriguing question is why, in view of the large spread of ee values using different sequences, it is possible to achieve >99% ee

Table 2 Sequence dependence of 1st and 2nd generation catalysts

	1st generation[a] ee (%)	2nd generation[b] ee (%)
st-DNA	37	98.5
Poly(dA-dT)	6	15 (−)
Poly(dG-dC)	62	78
$d(GCGCGCGCGCGC)_2$	54	95
$d(GCGCGCGC)_2$	27	86
$d(GACTGACTAGTCAGTC)_2$	34	78
$d(TCGGGTACCCGA)_2$	16	98.6
$d(TCAGGGCCCTGA)_2$	10	99.4

Conditions: all experiments were carried out with the following reagents: 0.3 mM [Cu(**L**)$(NO_3)_2$], 1.3 mg ml^{-1} st-DNA, 1 mM aza-chalcone, 16 mM cyclopentadiene, 20 mM MOPS pH 6.5, 5 °C, 3 days. In all cases the (+)-enantiomer was observed, unless noted otherwise.[a] With **L3**. [b] With **L9**.

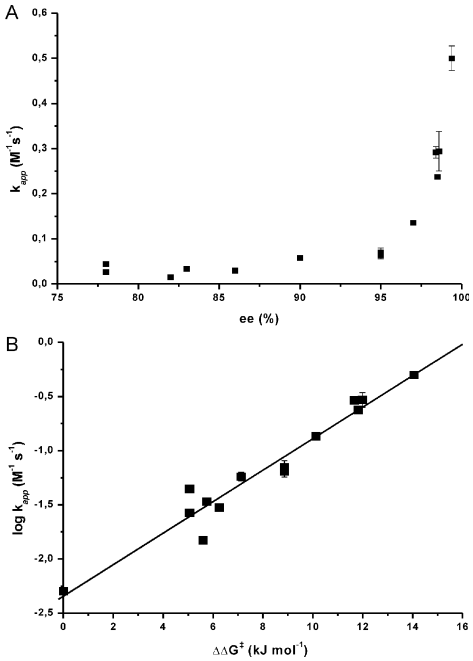


Fig. 2 (a) Relation between ee and k_{app}, and (b) between log k_{app} and $\Delta\Delta G^{\ddagger}$ in the DNA/Cu-dmbipy catalyzed Diels–Alder reaction of aza-chalcone with cyclopentadiene.

with st-DNA. For all sequences the binding affinity K_b was measured and it was found that there is no apparent sequence selectivity for binding of the copper complex. Hence, with st-DNA, the copper complex can be considered to be distributed evenly over the DNA, which means that the complexes reside in different micro-environments, which in turn induce different ee's. The answer was found in a kinetic study of the catalytic reaction in the presence of the oligonucleotides: with those sequences that gave rise to the highest ee' the largest rate accelerations were also observed (Fig. 2a). Indeed, a linear relation was found between the ee, expressed in $\Delta\Delta G^{\ddagger}$, and the log of the apparent 2nd order rate constant k_{app} (Fig. 2b).

Taken together these results explain why >99% ee can be achieved with st-DNA/Cu-dmbipy even though not all copper complexes are bound and the catalyst is actually a heterogeneous mixture of catalysts that reside in a different environment: the reaction is accelerated by DNA, and the DNA sequences that give the highest ee dominate the outcome of the reaction because they accelerate the reaction the most. In other words, the observed ee is not the average of all contributing DNA sequences, but the weighted average. These results are intriguing, because in asymmetric catalysis too often it is assumed that high (enantio-)selectivity can only be achieved at the expense of activity. Here the opposite is demonstrated: high enantioselectivity and high activity can be achieved simultaneously.

In a preliminary investigation of the Friedel–Crafts alkylation of **1b** with 5-methoxyindole, the same trends were observed. The apparent rate constant k_{app} of the Friedel–Crafts reaction increased 30-fold in the presence of DNA, compared to the Cu-dmbipy catalyzed reaction in the absence of DNA. This explains the catalyst loadings as low as 0.3 mol% that could be

used in some of these reactions without observing a decrease in enantioselectivity. Furthermore, the enantioselectivity of the Friedel–Crafts reaction proved to be dependent on the DNA sequence. Interestingly, a different sequence dependence was found for **1b** compared to **1c**,[29] which suggests that the optimal structure of the micro-environment provided by the DNA is substrate dependent.

Comparison between first and second generation catalysts

When comparing both generations of DNA-based catalysts, marked differences are observed. It is clear that, to date, the highest enantioselectivities and catalytic activities are observed with the second generation catalysts and in particular with DNA/Cu-dmbipy. This implies that applications in organic synthesis are more likely to be found with this class of DNA-based catalysts.

Interesting from a fundamental and mechanistic perspective is the observation that the effect and role of DNA in both generations of catalysts is different. Whereas with the first generation of catalysts the role of DNA is limited to that of a chiral scaffold, in the second generation DNA actively participates in the reaction, causing significant rate acceleration. Moreover, both generations of catalysts have different requirements for the DNA sequence to achieve the highest enantioselectivity. This is most likely related to the need for a differently structured DNA micro-environment to achieve high enantioselectivity. A final noteworthy difference is that both enantiomers of the product can be accessed using the first generation of catalysts, whereas with the second generation catalysts it is always the same enantiomer that is obtained in excess. In this regard the design of the first generation ligands is more versatile than that of the second generation.

A tentative explanation for the observed differences is that the catalytic metal centre is located in a different micro-environment depending on the type of ligand used. Due to the design, ligands of the 1st generation most likely cause the Cu^{2+} ion to be at the edge of the DNA. Hence, the catalytic reaction more resembles the reaction observed in the absence of DNA. In contrast, with the second generation of catalysts the reaction will most likely take place within the DNA groove where it is very sensitive to the structure and chemical properties of the micro-environment. As a result, DNA greatly influences the reaction in terms of activity and enantioselectivity.

The origin of enantioselectivity in DNA-based catalysis

The absolute configuration of the benchmark products of all three reaction types was determined, either directly or by conversion to a derivative with a known absolute configuration. The latter was achieved by treatment of the product with methyl triflate, followed by reaction with a nucleophile such as methanol, furnishing the corresponding methyl ester. Interestingly, it was found that the stereochemistry is predictable in the case of the st-DNA/Cu-dmbipy catalyst: the diene or nucleophile always attacks from the same π-face of the enone moiety, that is, the *Si* face in case of R_1 = aryl, and the *Re* face in case of R_1 = alkyl (Fig. 3). Therefore, it is likely that the enantiodiscrimination in these reactions is the result of the same mechanism.

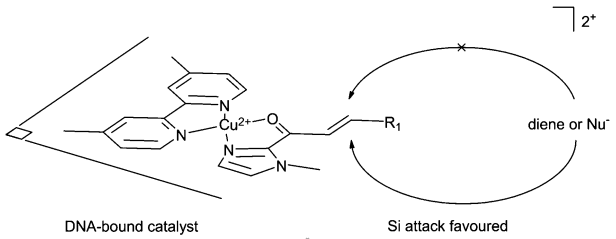


Fig. 3 Stereochemistry of the approach of the diene or nucleophile.

This could suggest that one face of the enone moiety is shielded by the DNA, resulting in preferred attack from the other face. However, shielding would likely give rise to a decrease of the reaction rate and this was not observed. On the contrary, in the Diels–Alder reaction the rate of formation of the favored enantiomer increased significantly whereas the rate of formation of the minor enantiomer was the same as was observed in the absence of DNA. These seemingly contradictory observations are tentatively explained by assuming an active role of the DNA environment in directing the incoming diene or nucleophile to the preferred π-face of the enone.

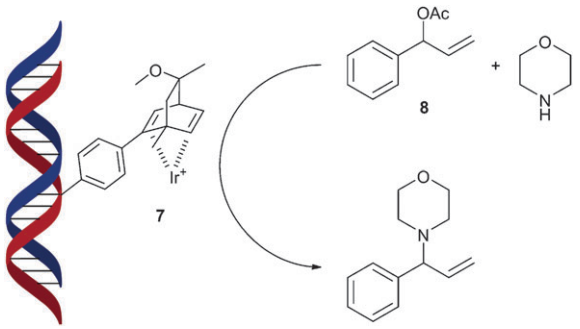
Covalent DNA-based catalysis approaches

Compared to supramolecular anchoring, covalent attachment of a catalytic moiety to DNA potentially offers some advantages. Using covalent anchoring, in principle, the greatest control over the micro-enviroment provided by the DNA to the catalyzed reaction can be achieved. This allows for a more rational approach to the design of DNA-based catalysts.

To date, the covalent approaches have focused mainly on the functionalization of nucleobases with a ligand that can bind a transition metal (Scheme 1). In initial attempts, a series of phosphine modified nucleotides and oligonucleotides have been prepared by reaction of activated ester containing phosphines with amino modified oligonucleotides. However, no catalysis was reported.[37] In a similar approach, a phosphine modified nucleotide was prepared and evaluated in the Pd(II) catalyzed allylic amination. Enantioselectivities of up to 82% ee were found in THF.[38] Incorporation in trinucleotides, as well as the presence of water in the catalytic reaction, lead to a lower reactivity and a loss of ee.

Jäschke and co-workers have recently reported an elegant design involving a diene ligand that was attached covalently to DNA.[39] The corresponding Ir-complex **7** proved to be an efficient catalyst for the allylic amination of **8** with morpholine, resulting in a kinetic resolution of **8** (Scheme 5). Although the enantioselectivities obtained are still modest, it is encouraging that the enantiomeric outcome of the reaction, that is, which enantiomer is found in excess, proved to be sensitive to the nature and structure of the polynucleotide scaffold. This suggests that the Ir-based system is a promising starting point for the development of enantioselective DNA-based organometallic catalysts.

An alternative approach involves the incorporation of metal binding non-nucleoside building blocks. Early reports on this approach involved the incorporation of metal salen complexes in double stranded DNA by templated synthesis.[40]



Scheme 5 Schematic representation of DNA-based Ir-catalyzed allylic amination.

These DNA-based catalysts were used only to achieve oxidative DNA strand scission.[41] Recently, a polyaza crown ether was incorporated in single stranded DNA. Upon hybridization with a complementary DNA strand and binding of Cu^{2+}, this DNA-based catalysts was evaluated in the asymmetric Diels–Alder reaction of aza-chalcone with cyclopentadiene.[42] However, only a low enantioselectivity of 10% was obtained.

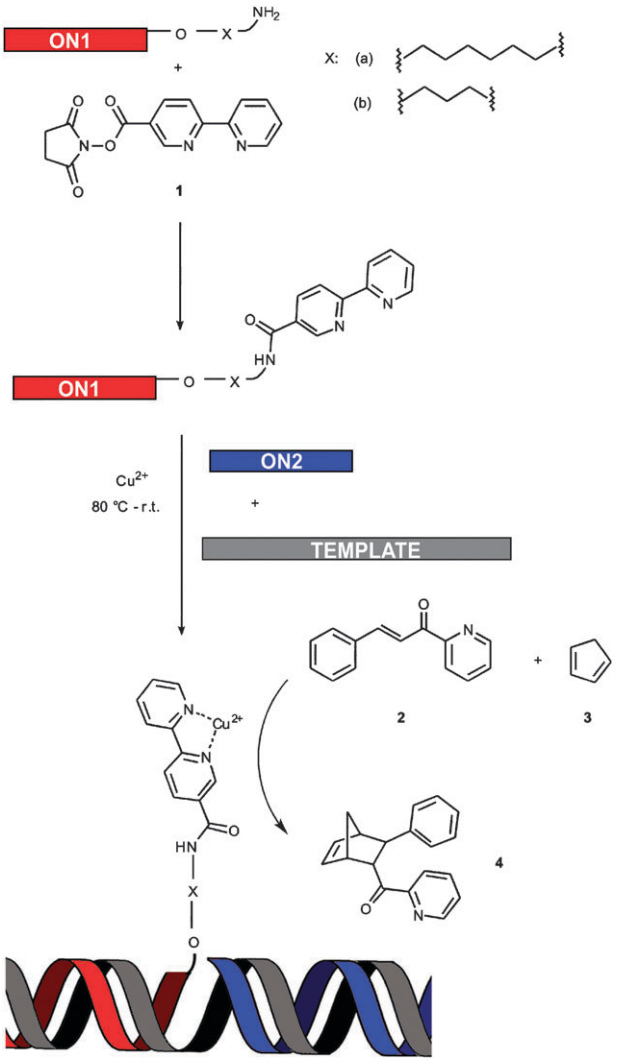
Recently, a modular approach towards catalyst assembly was reported, which makes use of 5′ and 3′ terminally modified oligonucleotides that are readily available from commercial sources.[43] This strategy involves three oligonucleotides, one of which has a covalently linked catalyst at its terminus. By judicious choice of sequences these oligonucleotides can be made to assemble into a duplex structure, which has the catalyst positioned internally at the interface between two oligonucleotides (Scheme 6). The advantage of this approach is that individual modules are readily exchanged, which obviates the need for synthesis of new oligonucleotide catalyst conjugates. The result is that the catalyst is optimized readily.

This concept was applied to the Diels–Alder reaction of aza-chalcone and cyclopentadiene. Using a bipyridine functionalized oligonucleotide **ON1**, and depending on the sequences of **ON2** used, ee's of up to 93% were obtained. It was found that sequences containing a G triplet did not induce the highest enantioselectivity, which indicates that a different DNA binding geometry of the copper complex to the DNA was active compared to the non-covalent attached copper complexes **L5–L9**. Instead, the triplets GTA and TAC in the template strand that are flanking the interface between ON1 and ON2 were found to induce the highest ee.

Miscellaneous approaches

In addition to DNA-based asymmetric catalysis, a number of alternative strategies have emerged for the application of DNA in catalysis, and can potentially be used for asymmetric catalysis.

DNAzymes, consisting of a DNA strand folded into catalytically active tertiary structures are promising catalysts for enantioselective synthesis.[44] Particularly attractive is that different topologies are available by varying the DNA sequence and the conditions, and that catalysts can be optimized using *in vitro* selection procedures. However, to date the catalytic scope of DNAzymes has been limited mainly to



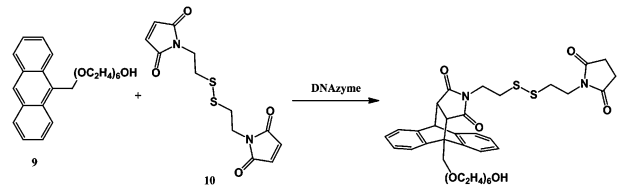
Scheme 6 Schematic representation of the assembly of a covalently anchored DNA-based catalyst and general reaction scheme.

(poly-)nucleotide chemistry, which is inherent to the selection methodologies that are commonly used.

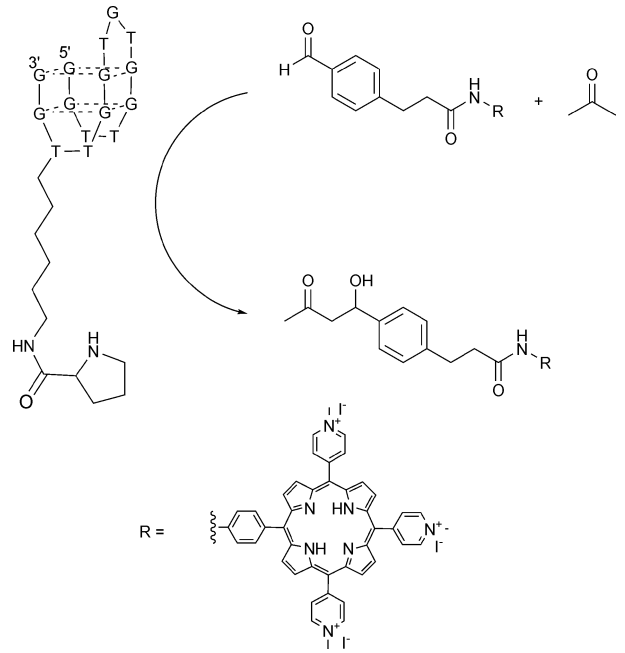
Two examples suggest that the scope of DNAzymes can be broadened to non-nucleotide reactions. A DNAzyme containing a hemin prosthetic group has been used as a catalyst in (per-)oxidation reactions with hydrogen peroxide. Although no enantioselective catalysis was reported, it was shown that the DNAzyme exhibited a small degree of diastereomeric selection of substrates.[45]

Recently, a DNAzyme capable of catalyzing the Diels–Alder reaction of **9** with **10** has been developed (Scheme 7).[23] The enantioselectivity of this reaction has not been reported to date. However, experiences with related RNAzymes, which give rise to ee's of up to 90%,[17] suggest that with DNAzymes enantioselective Diels–Alder reactions should also be feasible.

DNA-templated synthesis is the concept in which the reagents of a bimolecular reaction are linked to an oligonucleotide and brought together by a template DNA strand containing the complementary sequences to both oligonucleotides.[46,47] The result is a high effective molarity of the reactive groups, leading to efficient reactions at concentrations down to the



Scheme 7 Schematic representation of a DNAzyme catalyzed Diels–Alder reaction.



Scheme 8 Aldol reaction catalyzed by a DNA quadruplex-proline conjugate.

nanomolar range. In a variation of DNA-templated synthesis, proline modified DNA's have been used to catalyze aldol reactions.[48] In a first design an oligonucleotide linked benzaldehyde substrate was reacted with acetone to give multiple turnovers towards the aldol product. As a result of the covalent link of the substrate to the chiral DNA, in principle this could be a diastereoselective process, albeit no mention was made of the diastereomeric excess in the product. In an alternative design the benzaldehyde substrate was attached covalently to an achiral DNA quadruplex binding porphyrin.[49] The porphyrin bound and stabilized the G-quadruplex conformation of a DNA strand that contained proline (Scheme 8). The close proximity of the proline and benzaldehyde reactant resulted in efficient aldol reaction with acetone. In this case none of the reactants were linked covalently to DNA, which means that enantioselective catalysis is possible. However, to date the enantioselectivity of this reaction has not been reported.

Conclusions and outlook

DNA has emerged as an attractive scaffold for the design of novel enantioselective catalysts. Of the two approaches developed, in particular the supramolecular approach has given rise to DNA-based catalysts capable of inducing enantioselectivity in a variety of Lewis acid catalyzed reactions. Attractive features of this approach include the modular assembly of the catalyst, which allows for rapid optimization, and the fact that DNA in several cases helps to accelerate the reaction. The latter point raises interesting questions about the role of the second coordination sphere provided by the DNA, which may hold important clues for our understanding of (bio-)catalysts and their design. This methodology is now at the stage where applications in synthesis can be envisioned. Indeed, the first examples demonstrate that these reactions can be competitive with their "conventional" analogues, both in terms of practicality and cost.

DNA-based catalysts containing covalently linked catalytic moieties potentially offer greater control over the geometry of the catalyst and the second coordination sphere. However, these approaches are still in their infancy and many obstacles, not least the major synthetic effort that goes into the preparation of such catalysts, need to be overcome. Based on the results presented in this review and in view of the versatility of DNA as a chiral scaffold for design, it can be concluded that DNA-based asymmetric catalysis is rapidly emerging as a promising new concept in catalysis.

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